# OCCURRENCE OF SEED FUNGI FROM 37 LOBLOLLY SEEDLOTS COLLECTED IN 19 SEED ORCHARDS

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## ABSTRACT

Pathogenic fungi (*Fusarium* spp., *Fusarium moniliforme* var. *subglutinans*, and *Diplodia* spp.) were found commonly on the surface of loblolly pine seed in most of the lots screened. These fungi were effectively controlled with hydrogen peroxide. Only 2 of 37 seedlots had enough internal seed fungi to be of any concern. This demonstrates that proper screening, coupled with proper cultural techniques, can reduce the amount of pathogenic fungi placed in nursery seedbeds.

## INTRODUCTION

Southern forestry has become increasingly dependent on a continuing supply of pine seed produced from trees grown in intensively managed seed orchards. The cost of producing this seed is high because of the variety of cultural practices used to obtain maximum production. Insects have been recognized as causing major seed losses. Seed fungi have long been recognized as a problem in agronomic crops. In 1977, Miller and Bramlett reported that pathogenic fungi were damaging slash pine seed. This led to a survey of seed grown in slash pine seed orchards in the South (Anderson, Belcher, Miller, and Martin). All of the seedlots examined in this survey had some fungi in the internal tissue; i.e., gametophyte tissues in the embryo. About 10 percent of the sound seed and 15 percent of the unsound seed contained potentially pathogenic internal fungi. *Diplodia* sp. was the most common pathogenic fungus isolated, followed by *Fusarium* sp. and *Fusarium moniliforme* var. *subglutinans*. In one of the 2 years, the pitch canker fungus was not recovered. This shows that there can be year-to-year variability in the occurrence of seedborne fungi.

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Because of the results of the slash pine survey, a 2-year survey was planned to assess the occurrence of fungi in the seed of loblolly pine. This report presents the results for the first year. Unlike the slash pine survey, loblolly seed were examined for external as well as internal fungi.

## METHODS

Thirty-seven seedlots of loblolly pine from 19 seed orchards in 8 states were examined. One hundred and fifty seed were sampled from each seedlot submitted. Each seedlot was divided into three 50-seed treatment groups. In Treatment 1, whole seeds were plated onto acidified potato dextrose agar (A-PDA). In Treatment 2, whole seeds were plated onto A-PDA after being soaked 10 minutes in 20 percent hydrogen peroxide and then rinsed with sterile, distilled water. In Treatment 3, the whole seed was soaked in 70 percent ethyl alcohol for 10 minutes. One-half of the gametophyte tissue was soaked in 10 percent chlorox for 10 minutes, rinsed with sterile, distilled water, and plated onto A-PDA (the agar was acidified to a pH of 4.7 $\pm$ .1, with 50% lactic acid).

These seed were separated as sound and unsound. A sound seed looked normal inside, while unsound gametophyte tissue appeared discolored or dried up. Each seed was examined frequently, starting three days after plating and continuing for two weeks or until a fungus was identified. Even though some seed had more than one fungus per seed, only one fungus was reported per seed. Known pathogenic fungi (*Diplodia* sp., *Fusarium* sp., and *Fusarium moniliforme* var. *subglutinans*) were given priority, followed by suspected pathogenic fungi, and, finally the most common fungus in selecting which fungus to report.

## RESULTS

Of the untreated, complete seed plated (Treatment 1), over 99 percent had fungi on the seed coat (Table 1). Pathogenic fungi were recovered from the seed coats in 31 of the 37 seedlots. Eighty-one percent of the seedlots had *Fusarium* spp.; 18 percent had *Fusarium moniliforme* var. *subglutinans*; and 27 percent had *Diplodia* spp. Thirty-eight other species of fungi were isolated from this treatment (Table 2).

In most cases, the hydrogen peroxide treatment (Treatment 2) reduced the number of fungi isolated. The number of pathogenic fungi isolated were reduced in 80 percent of the seedlots (Table 1). Twenty percent of these seedlots remained the same as far as the three pathogenic fungi are concerned, or showed a slight increase. Over 40 other species of fungi were isolated from these seed (Table 2).

Few fungi were isolated from the internal tissues of the seed (Treatment 3). Only 24 percent of the seedlots with sound seed had any internal pathogenic fungi. Internal pathogenic fungi were present in less than 1 percent of the seed in these (24) lots. Unsound seed had even fewer pathogens. For Treatment 3, the number of seed with any fungi averaged between 5 and 8 percent (Table 1).

Table 1.--Occurrence of pathogenic and non-pathogenic fungi on the surface of seed from 37 loblolly seedlots (Treatment #1), after treatment with hydrogen peroxide (Treatment #2), and internal fungi (Treatment #3), 1982.

LOBLOLLY SEED FUNGI SURVEY, 1981-1982

| Seed Orchard No. | Treatment #1 | | | | | Treatment #2 | | | | | Treatment #3 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Sound Seed | | | | | Unsound Seed | | | | | | |
| | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Sound | % Unsound |
| | 1 | 2 | 3 | | | 1 | 2 | 3 | | | 1 | 2 | 3 | | | 1 | 2 | 3 | | | | |
| 1 | 12 | 10 | 6 | 72 | 100 | 12 | - | 2 | 64 | 78 | - | - | 2 | - | 2 | - | - | - | - | - | 98 | 2 |
| 1 | 8 | - | - | 92 | 100 | 2 | - | - | 70 | 72 | 2 | - | - | 2 | 4 | - | - | - | - | - | 96 | 4 |
| 2 | - | - | - | 100 | 100 | - | - | 4 | 38 | 42 | - | - | - | 4 | 4 | - | - | - | - | - | 98 | 2 |
| 2 | - | - | - | 100 | 100 | 10 | - | - | 60 | 70 | - | - | - | 2 | 2 | - | - | - | - | - | 94 | 6 |
| 3 | 10 | - | 2 | 88 | 100 | 2 | - | - | 98 | 100 | - | - | - | 9 | 9 | - | - | - | 13 | 13 | 70 | 30 |
| 4 | 38 | 28 | - | 34 | 100 | - | - | - | 58 | 58 | - | - | - | 3 | 3 | - | - | - | - | - | 80 | 20 |
| 4 | - | - | - | 100 | 100 | - | - | - | 40 | 40 | - | - | - | 5 | 5 | - | - | - | - | - | 84 | 16 |
| 5 | 10 | - | - | 90 | 100 | - | - | - | 4 | 4 | - | - | - | 3 | 3 | - | - | - | - | - | 80 | 20 |
| 5 | 4 | - | - | 96 | 100 | - | - | - | 100 | 100 | - | - | - | 6 | 6 | - | - | - | - | - | 32 | 68 |
| 6 | 8 | - | - | 92 | 100 | - | - | 2 | 78 | 80 | - | - | - | 4 | 4 | - | - | - | - | - | 98 | 2 |
| 6 | 32 | 8 | - | 60 | 100 | - | - | - | 62 | 62 | - | - | - | - | - | - | - | - | - | - | 100 | 0 |
| 7 | - | - | - | 100 | 100 | - | - | - | 58 | 58 | - | - | - | - | - | - | - | - | - | - | 74 | 26 |
| 7 | - | - | - | 100 | 100 | 4 | - | - | 44 | 48 | - | - | - | 2 | 2 | - | - | 2 | - | 2 | 90 | 10 |
| 8 | 22 | 8 | - | 70 | 100 | - | - | - | 20 | 20 | - | - | - | 2 | 2 | - | - | - | 100 | 100 | 98 | 2 |
| 8 | 12 | 12 | 2 | 74 | 100 | - | - | - | 6 | 6 | - | - | - | 6 | 6 | - | - | - | - | - | 100 | 0 |
| 8 | 2 | - | - | 94 | 96 | - | - | - | 10 | 10 | - | - | - | - | - | - | - | - | 17 | 17 | 94 | 6 |
| 9 | 2 | - | - | 96 | 98 | 2 | - | - | 42 | 44 | - | - | - | 2 | 2 | - | - | - | - | - | 96 | 4 |
| 9 | 6 | - | 2 | 92 | 100 | 4 | - | 2 | 32 | 38 | - | - | - | 6 | 6 | - | - | - | - | - | 98 | 2 |
| 10 | 26 | - | - | 72 | 98 | - | - | 6 | 44 | 50 | - | - | - | - | - | - | - | - | - | - | 98 | 2 |
| 10 | 16 | - | - | 84 | 100 | 8 | - | 2 | 90 | 100 | 2 | - | - | 4 | 6 | - | - | - | - | - | 100 | 0 |

KEY

1 - Fusarium sp.
2 - Fusarium moniliforme var. subglutinans
3 - Diplodia sp.

Table 1.--Con't

| Seed Orchard No. | Treatment #1 | | | | | Treatment #2 | | | | | Treatment #3 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Sound Seed | | | | | Unsound Seed | | | | | | |
| | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Known Pathogens | | | % Other Fungi | Total % Fungi | % Sound | % Unsound |
| | 1 | 2 | 3 | | | 1 | 2 | 3 | | | 1 | 2 | 3 | | | 1 | 2 | 3 | | | | |
| 11 | 56 | - | - | 44 | 100 | 26 | - | 2 | 68 | 96 | 14 | - | 3 | 6 | 23 | - | - | 7 | - | 7 | 70 | 30 |
| 11 | 62 | 4 | 6 | 28 | 100 | 2 | - | 2 | 26 | 30 | - | - | - | 7 | 7 | - | - | - | - | - | 92 | 8 |
| 12 | 16 | 48 | 2 | 34 | 100 | - | - | 2 | 60 | 62 | - | - | - | - | - | - | - | - | - | - | 78 | 22 |
| 13 | 34 | 16 | 8 | 42 | 100 | 14 | - | - | 78 | 92 | 2 | - | - | 5 | 7 | - | - | - | - | - | 88 | 12 |
| 13 | 40 | 6 | 12 | 42 | 100 | - | - | - | 94 | 94 | - | - | - | - | - | - | - | - | - | - | 100 | 0 |
| 14 | 62 | 16 | - | 22 | 100 | - | - | - | 56 | 56 | - | - | - | - | - | - | - | - | - | - | 100 | 0 |
| 14 | 20 | 2 | - | 78 | 100 | 12 | - | - | 88 | 100 | 4 | - | - | 2 | 6 | - | - | - | - | - | 100 | 0 |
| 15 | 12 | 14 | - | 74 | 100 | 18 | 2 | - | 80 | 100 | 4 | 2 | - | 2 | 8 | - | - | - | - | - | 100 | 0 |
| 15 | 48 | 8 | 2 | 42 | 100 | 16 | 4 | - | 68 | 88 | 6 | 4 | 2 | - | 12 | - | - | - | - | - | 100 | 0 |
| 16 | 16 | - | - | 84 | 100 | - | - | - | 18 | 18 | - | - | - | - | - | - | - | - | - | - | 100 | 0 |
| 16 | 18 | 20 | - | 62 | 100 | - | - | - | 80 | 80 | - | - | - | 6 | 6 | - | - | - | - | - | 94 | 6 |
| 17 | - | - | - | 100 | 100 | 6 | - | - | 94 | 100 | - | - | - | 14 | 14 | - | - | - | 100 | 100 | 98 | 2 |
| 17 | 4 | - | - | 96 | 100 | 4 | - | 4 | 92 | 100 | - | - | - | 50 | 50 | - | - | - | 38 | 38 | 84 | 16 |
| 18 | 6 | - | - | 94 | 100 | - | - | - | 96 | 96 | 2 | - | - | 4 | 6 | - | - | - | 20 | 20 | 90 | 10 |
| 18 | 10 | 2 | - | 88 | 100 | 2 | - | - | 98 | 100 | - | - | - | 8 | 8 | - | - | - | - | - | 100 | 0 |
| 19 | 92 | 6 | - | 2 | 100 | 2 | - | - | 78 | 80 | - | - | - | 2 | 2 | - | - | - | - | - | 100 | 0 |
| 19 | 82 | 10 | - | 8 | 100 | - | - | - | 68 | 68 | - | - | - | - | - | - | - | - | - | - | 100 | 0 |
| Avg. | 21 | 11 | 1 | 72 | 100 | 4 | 0 | 1 | 61 | 66 | 1 | 0 | 0 | 5 | 6 | 0 | 0 | 0 | 8 | 8 | 91 | 9 |

Table 2.--Genera of fungi isolated from 37 loblolly seedlots, by type of isolation technique, 1982.

| Fungus | Treatments 1/ | Fungus | Treatments |
|---|---|---|---|
| Acrospeira spp. | 1,2 | Metarrhizium spp. | 1 |
| Alternaria spp. | 1,2 | Monilia spp. | 1 |
| Ascomycete | 2 | Monocillium spp. | 2,3 |
| Aspergillus spp. | 1,2,3 | Monotospora spp. | 2 |
| Asteromella spp. | 2 | Nigrospora spp. | 1,2,3 |
| Aureobasidium spp. | 1,2 | Nodulisporium spp. | 1,2 |
| Bacterium | 1,2,3 | Oidium spp. | 2 |
| Basidiomycete | 1,2,3 | Olpitrichum spp. | 1 |
| Bispora spp. | 1,2 | Papulospora spp. | 1,2 |
| Candida spp. | 2 | Penicillium spp. | 1,2,3 |
| Cephalosporium spp. | 1,2 | Pestalotia spp. | 1,2,3 |
| Chaetomium spp. | 2 | Phomopsis spp. | 2 |
| Chaetophoma spp. | 1 | Phycomycete | 3 |
| Chlamydomyces spp. | 1,2 | Powdery Mildew | 1,2 |
| Cladosporium spp. | 1,2,3 | Rhizopus spp. | 1,2,3 |
| Curvularia spp. | 1 | Sphaeronaema spp. | 1 |
| Dendrophoma spp. | 2 | Spondylocladium spp. | 1 |
| Epicoccum spp. | 2 | Sporothrix spp. | 1 |
| Erysiphe spp. | 2 | Sporotrichum spp. | 1,2 |
| Geotrichum spp. | 3 | Staphylotrichum spp. | 2 |
| Gilmaniella spp. | 1,2 | Streptomycete | 2 |
| Gliocladium spp. | 2 | Tetracoccosporium spp. | 1 |
| Gonatobotrys spp. | 2 | Torula spp. | 1 |
| Gonatobotryum spp. | 1 | Trichaegum spp. | 1 |
| Hansfordia spp. | 2,3 | Trichoderma spp.** | 1,2,3 |
| Helminthosporium spp. | 1 | Trichothecium spp. | 1,2 |
| Humicola spp. | 1,2 | Tritirachium spp. | 1 |
| Hyalodendron spp. | 2 | Umbelopsis spp. | 3 |
| Isaria spp. | 2 | Verticillium spp. | 2 |
| Melanospora spp.* | 1,2 | Yeast | 1,2,3 |

1/ 1 = plate whole seed
2 = plate whole seed after hydrogen peroxide treatment
3 = remove and plate endosperm

* May be the same as Humicola.

** May be the same as Trichothecium.

Some seedlots had no pathogenic fungi on the seed coat or within the internal tissue. Others, such as the first seedlot in seed orchard No. 19, had 98 percent of the seed with pathogens on the surface, but only 2 percent after hydrogen peroxide treatment and none inside of the seed. In the first seedlot in seed orchard No. 11 Fusarium spp. were recovered both from untreated and treated seed and from internal tissues (Table 1).

## DISCUSSION

The presence of seedborne pathogens in seed orchards is so new, we are not sure of the total significance of the problem to southern forestry. However, there are several conclusions that can be drawn from these data.

The isolation of fungi from the untreated seed (Treatment 1) was expected, but not with the high frequency that occurred. Eighty-four percent yielded Fusarium spp., with frequencies as great as 92 percent. The pitch canker fungus (FMS) was present on 46 percent of the seedlots, with individual lots having up to 48 percent of seed producing FMS. These fungi, with the proper conditions, can cause damping-off, root rot, and pitch canker. Placing such untreated seed in a nursery bed is like inoculating the bed with pathogenic fungi. Treatment 2 shows that most of these fungi can be controlled with minimal seed treatment. This survey was not designed to examine types of seed treatment. Hydrogen peroxide works, and other chemicals, such as fungicides, may provide even better control.

Treatment 3 demonstrates that the occurrence of internal seed fungi in loblolly pine is low. Slash pine averaged about 15 percent internal fungi, while these loblolly seedlots (sound seed) averaged less than 1 percent with internal fungi. The unsound seed contained few fungi, but most of these seed were empty rather than damaged. In the second year of this survey, records of unsound and empty seed will be separated.

The important point to note is that all seedlots cannot be viewed as the same. By screening, as has been done here, the types of cultural treatments needed can be tailored to the individual seedlot.

These types of data could be used in a number of ways. Some are listed below.

1. To identify for seed orchard managers those seedlots with pathogenic seed fungi.

2. To allow the manager to favor less infected seedlots (over heavily infected ones) when there is an excess seed supply.

3. To identify seedlots that have a high occurrence of pathogenic fungi restricted to the unsound seed and remove the unsound seed.

4. To allow orchards to regulate the shipment of seed containing pathogenic fungi between or within states or to foreign countries.

5. To allow managers to closely monitor those nursery beds seeded with seedlots known to contain pathogenic fungi; this will reduce detection delay should a disease problem occur.

6. To examine the influence of cultural practices, such as methods of extraction and storage, on the abundance of pathogenic fungi on and inside of the seed.

## REFERENCES

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